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# Studies on the Mechanisms of Detoxication of Histamine by Methylmethionine Sulfonium Chloride

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1. To study the mechanisms of inactivation of histamine,  $^{14}\text{CH}_3\text{-MMSC}^{**}$  was administered orally and an hour later, histamine was injected intraperitoneally. N-methylhistamine, $^{***}$  inactive form of histamine, was isolated from urine. In 24 hours the methyl group of MMSC transferred to the histamine with one-fourth of the dilution on specific activity. From these data, MMSC appears to be a very effective methyl donor for histamine methylation.

2. The distribution of isotope in tissues and excreta of female mice has been studied after the oral administration of radioactive MMSC. About 30 per cent of the radioactivity,  $^{14}\text{C}$  in position of either methyl group or carboxyl group, was excreted in the urine within 24 hours. The radioactivity in the tissues was recovered as MMSC and methionine in the liver, intestine and stomach.

## INTRODUCTION

It is well known that S-methylmethionine sulfonium chloride (MMSC) is a natural constituent of some plant tissues<sup>1)</sup>; and the metabolisms of MMSC in animals<sup>2)</sup>, microorganisms<sup>3)</sup>, and plants<sup>4)</sup> have already been studied by several groups of workers. MMSC, known to have a high energy bond of the methyl group, has more recently been implicated to be the biological methyl donor in mammalian enzymatic transmethylation<sup>5,6)</sup>.

One of the metabolic pathways of MMSC is to be decomposed to homoserine and dimethylsulfide<sup>7)</sup>, and the other is, by the transfer of one of the methyl groups to homocysteine, to turn into two moles of methionine<sup>8)</sup>. The metabolic fates of MMSC *in vivo*, however, have not been known completely yet.

Recently MMSC has come to be used in the treatment of peptic ulcer. Though its effect has not been confirmed yet, it can be considered that MMSC is a very effective methyl donor to histamine, which is known to produce gastric ulcer experimentally. 1, 4-Methylhistamine, $^{***}$  methylated histamine, is an inactive form of administered histamine in most species including man, and the extremely high amount of methylhistamine has been found in female mice<sup>9)</sup>.

In the present paper, one of the mechanisms of detoxication of histamine is described as MMSC changes into methionine, and the methyl group of methionine can be utilized for the methylation of histamine.

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\*\* MMSC=methylmethionine sulfonium chloride

\*\*\* N-methylhistamine=1, 4-methylhistamine=1-methyl-4-( $\beta$ -aminoethyl)-imidazole

## EXPERIMENTAL PROCEDURE

**Materials**— $^{14}\text{CH}_3$ -Methylmethionine sulfonium iodide and  $^{14}\text{COOH}$ -methylmethionine sulfonium iodide were obtained from Daiichi Pure Chemicals, Tokyo. Methylhistamine was synthesized by the method of Rothschild and Schayer<sup>10</sup>. Other chemicals were of the highest purity available from commercial sources.

**Preparation of radioactive MMSC**—Into 0.05 ml of water, 20 mg of  $^{14}\text{C}$ -MMSI (3.4 mc/m mole of  $^{14}\text{COOH}$ -MMSI or 2.3 mc/m mole of  $^{14}\text{CH}_3$ -MMSI) were dissolved. To this solution, 0.15 ml of colloidal  $\text{AgCl}$ , prepared from 20 mg of  $\text{AgNO}_3$  immediately before use, was added, then the mixture was shaken well for 3 hours at room temperature. After centrifugation, the supernatant was passed through Amberlite CG-50 column ( $\text{OH}^+$  form,  $1 \times 10$  cm) and MMSC was eluted with water. Fraction of MMSC was concentrated and a granular precipitate was obtained by the addition of the mixture of methanol and acetone (1:1).

**Measurement of Radioactivity**—Radioactivity of  $^{14}\text{C}$  was measured by the Packard Liquid Scintillation Spectrometer. To 0.2 ml of test solution, 10.0 ml of Bray's solution<sup>11</sup> were added and the sample was measured at 1080 V.  $^{14}\text{CO}_2$  was trapped in the 2N NaOH solution, transformed to  $\text{BaCO}_3$  by the addition of saturated  $\text{BaCl}_2$  solution, filtered, dried and counted with 0.3 g of CAL-O-SIL and 10.0 ml of Bray's solution.

**Determination of Methylhistamine**—Samples of urine were taken to at least pH 13 with 10N NaOH, saturated with NaCl, and shaken for 10 minutes with 1 volume of a solution containing equal parts by volume of *n*-butanol and chloroform. After centrifugation at  $10,000 \times g$  for 10 minutes, the upper layer was added to 1.5 volumes of heptane and 0.1 volume of 0.2 N HCl. The mixture was shaken for 5 minutes and then centrifuged at  $500 \times g$  for 2 minutes. The lower layer was dried under reduced pressure in a rotatory evaporator. The extract of the urine was dissolved in 0.1 ml of water and applied on the Whatman No. 3 MM filter paper. It was developed with the solvent of *t*-butanol-formic acid-water (70:15:15). At the  $R_f$  corresponding to N-methylhistamine ( $R_f=0.36$ ), the paper was cut and the spot was eluted with water. It was again developed by paperchromatography in the solvent system of *n*-propanol-28%  $\text{NH}_4\text{OH}$ - $\text{H}_2\text{O}$  (75:1.5:23.5). From the area corresponding to N-methylhistamine ( $R_f=0.75$ ), it was eluted with water and condensed to 1 ml. An aliquot (0.5 ml) was counted and another aliquot (0.5 ml) was assayed for methylhistamine by the Diazo reagent<sup>12</sup>.

**Histamine test**—Twenty mg of  $^{14}\text{CH}_3$ -MMSC ( $1.3 \times 10^6$  cpm) was administered by tubing method. After one hour, 0.1 ml aqueous solution of 1 mg histamine and 100  $\mu\text{g}$  aminoguanidine was injected intraperitoneally. Radioactive N-methylhistamine excreted in the urine during 24 hours was collected and its radioactivity was measured.

**Animals**—Eight female mice, each weighing approximately 30 g, were housed individually in closed glass bottles. Two mg of radioactive MMSC in 0.2 ml water solution were administered by tubing method.  $\text{CO}_2$  free air was passed on the closed animal bottle and expired  $\text{CO}_2$  gas was trapped in 2N NaOH.

## RESULTS

**Transmethylation of MMSC to histamine**—Four female mice orally received 20 mg of  $^{14}\text{CH}_3\text{-MMSC}$  ( $1.3 \times 10^6$  cpm), and an hour later, 1 mg of histamine was injected intraperitoneally. During 24 hours, 2552 cpm of radioactive N-methylhistamine was detected in the urine. The amount of obtained N-methylhistamine was 105  $\mu\text{g}$ . In this result, we recognized that methyl group of MMSC was transferred to the histamine with one-fourth of the dilution on specific activity. When  $^{14}\text{CH}_3\text{-methionine}$  was replaced by  $^{14}\text{CH}_3\text{-MMSC}$  in the experiment of transmethylation to histamine, the dilution of specific activity was about half of the former experiment. These results indicate that the methyl radicals of methionine should play as a methyl donor on the methylation of histamine.

Table I. Effect of  $^{14}\text{C}$ -MMSC on the biosynthesis of methylhistamine in mice.  $^{14}\text{C}$ -MMSC was administered orally. After one hour, 1 mg of histamine was injected intraperitoneally. N-Methylhistamine was isolated from urine and its radioactivity was measured.

RADIOACTIVITIES IN N-METHYLHISTAMINE

20 mg $^{14}\text{C}$ -MMSC ( $1.3 \times 10^6$ cpm)	1 mg HISTAMINE	0~24 hrs	24~48 hrs
		CPM	
+	+	2552	192
+	—	168	28

Table II. Effect of methionine on the biosynthesis of N-methylhistamine.

Conditions were same as described in Table I.

RADIOACTIVITIES IN N-METHYLHISTAMINE (0~24 hrs urine)

20 mg $^{14}\text{C}$ -MMSC ( $1.3 \times 10^6$ cpm)	20 mg $^{14}\text{C}$ -METHIONINE ( $1.4 \times 10^6$ cpm)	1 mg HISTAMINE	RADIOACTIVITY (cpm)
	+	+	3780
	+		120
+		+	2552

Table III. Distribution of  $^{14}\text{C}$ , 24 hours after administration of  $^{14}\text{C}$ -MMSC in mice.

Radioactive MMSC was administered orally in mice at a dose of 2 mg. The radioactivities of the administered dose recovered in the major fractions were summarized.

DISTRIBUTION OF  $^{14}\text{C}$  AFTER 24 HOURS

	$^{-14}\text{COOH}$ ( $85 \times 10^4$ CPM)		$^{-14}\text{C H}_3$ ( $55 \times 10^4$ CPM)	
	CPM ( $\times 10^4$ )	$^{14}\text{C}$ %	CPM ( $\times 10^4$ )	$^{14}\text{C}$ %
$\text{CO}_2$	23.5	27.5	0.3	0.5
Urine	23.7	28.5	13.5	27.0
Carcass	17.6	21.2	22.8	42.8

**Excretion of radioactive carbon after administration of  $^{14}\text{C}$ -MMSC**—After the oral administration of 2 mg of  $^{14}\text{COOH}$ -MMSC ( $85 \times 10^4$  cpm), expired radioac-

tive  $\text{CO}_2$  was trapped. From the results, summarized in Table III, it was seen that radioactivity of  $\text{CO}_2$  increased remarkably in 4 hours, whereas only a small amount of radioactive  $\text{CO}_2$  was detected by the administration of  $^{14}\text{CH}_3\text{-MMSC}$  ( $5 \times 10^6$  cpm).

**Distribution of MMSC in the body**—Four mice were orally given either 2mg of  $^{14}\text{COOH-MMSC}$  ( $85 \times 10^4$  cpm) or 2mg of  $^{14}\text{CH}_3\text{-MMSC}$  ( $55 \times 10^4$  cpm). They were killed by exsanguination 24 hours after the administration. As shown in Table III, in both cases about 28 per cent of radioactivities were found in urine and carcass. Of all the organs, intestine and liver contained particularly high radioactivities (Fig. 1). After the administration of 2 mg of  $^{14}\text{CH}_3\text{-MMSC}$  ( $5 \times 10^6$  cpm), radioactivities of some organs were measured by hours. In the stomach, as shown in Fig. 1, count decreased with time and after 2 hours, count dropped to  $4 \times 10^5$  cpm. Even in the intestine the same results were observed. But in the liver radioactivity increased during the first 4 hours as to  $7 \times 10^5$  cpm and then began to decrease slowly, but even after 48 hours a considerable amount of radioactivity were detected in these organs.

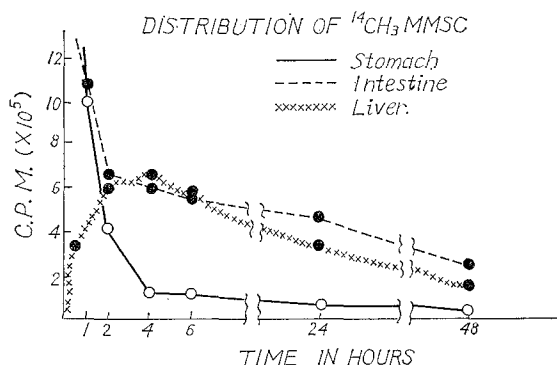


Fig. 1. Radioactivities of carbons following oral administration of  $^{14}\text{CH}_3\text{-MMSC}$ . Administered count was  $5 \times 10^6$  cpm and determination were made in duplicate on all samples.

To identify the radioactive compound, paperchromatography was carried out. Stomach was homogenized by Potter's homogenizer and deproteinized by the addition of trichloroacetic acid. Excess of trichloroacetic acid were extracted several times with ether, and pH was brought to at least 4. The volume was reduced to about 0.5 ml, and it was developed with the solvent of butanol, acetic acid and water (80:20:40). In this result, as shown in Fig. 2, radioactivities of MMSC and methionine were detected in the ratio of 7:1. To identify these compounds, two other solvent systems were used for paperchromatography. In these experiments  $R_f$  values of the obtained MMSC and methionine agreed with the authentic samples respectively. When cold methionine was added to the radioactive methionine as carrier, and recrystallized with water and acetone, no significant drop in specific activities was observed.

On the basis of these experiments, we concluded that a part of MMSC was metabolized to methionine and a large amount of unchanged MMSC remained in

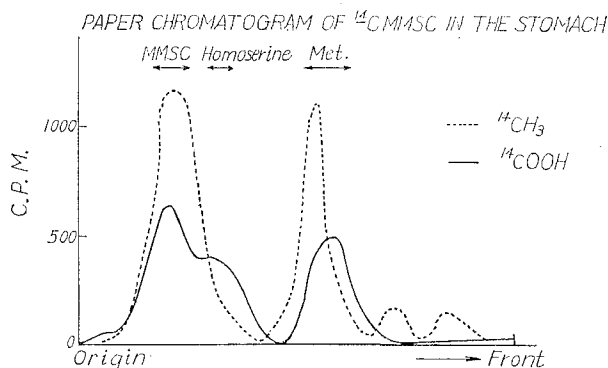


Fig. 2. Paperchromatogram of  $^{14}\text{C}$ -MMSC in the stomach. Conditions as described in the text. Stomach extract was condensed to 0.5 ml and developed with the solvent of butanol, acetic acid and water. Paper was cut into 1 cm strips and counted with 10 ml of Bray's solution by the Packard Liquid Scintillation Spectrometer.

the organs.

## DISCUSSION

Histamine is known to have a parasympathetic stimulating action and stimulate the secretion of gastric, pancreatic and salivatic juice. Therefore, histamine has been used to provoke experimental gastric ulcers and has been thought one of the causes of human gastric ulcer. So the inactivation mechanism of histamine *in vivo* seems very attractive problem for the investigation of therapeutic action on the gastric ulcer. A major pathway of histamine inactivation in mammals is the methylation of imidazole ring to yield methylhistamine. A possible pathway, which methylate histamine to utilize MMSC as a methyl donor, has been known to involve these reactions, *i.e.* MMSC→Methionine→S-Adenosylmethionine→N-Methylhistamine. In this pathway, each enzyme was isolated and its character, including substrate specificity, were clarified<sup>(12,13,14,15)</sup>. On the other hand, direct transmethylation from MMSC to histamine, or from MMSC to ATP was also possible, because the energy level of sulfonium radical of MMSC was very high (about 12 K Cal)<sup>(5)</sup>. The detailed experiments with liver and kidney homogenate are now under investigation.

When 100 mg of *L*-methionine were administered in men every day, GPT (glutamic-pyruvic transaminase) value of liver increased remarkably within 3 weeks, and fatty liver was often observed in three months<sup>(16)</sup>, whereas when 500 mg of *DL*-MMSC were administered for three months, any pathological change could not be detected in the liver.

Moreover, free methionine pool in liver is very small and only 1/500-1/1000 amount of methionine, contained in protein, is observed as free methionine<sup>(17)</sup>. In these results, MMSC seems to be able to be utilized for the very effective methyl donor on the methylation of histamine.

Many acceptors of methyl group in transmethylation reaction are known and

histamine is only one example of methyl acceptors. And the pathogenesis of gastric ulcer is not so simple. But the fact that MMSC can play a good methyl donor on the inactivation of histamine, seems to give one important key to the treatment of gastric ulcer.

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